Optimization and Automation of Artificial Tick Feeding

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"Our imagination is struck only by what is great; but the lover of natural philosophy should reflect equally on little things."

Alexander von Humboldt

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Abbreviations

Arg – Arginin

ATP – adenosine triphosphate

CCHF – Crimean Congo Haemorrhagic Fever

CO_2 – carbon dioxide

DCM – dichloromethane

FCS – fetal calf serum

FSME – tick borne encephalitis (Frühsommer-Meningoenzephalitis)

GSH - glutathion

Hb – hemoglobin

LVM/mL – low volatile mass/ milliliter(i.e. the mass of all the substances solved in 1 mL of extract)

M – Molar (= mol/ L)

NaCl – sodium chloride

$NaHCO_3$ – sodiumbicarbonate

mg – milligram

mL - milliliter

mOsm - Milliosmol

RH – relative humidity

RSSE – Russian Spring Summer Encephalitis

SAS – semi-automated system

TBE – tick-borne encephalitis

2,3-DPG – 2,3-diphosphoglycerate

Chapter 1: Introduction

About Ticks

Ticks (Ixodida) are acarine arthropods that can be divided into three families, the hard ticks (Ixodidae), the soft ticks (Argasidae) and a third taxon called Nuttalliellidae, which is embodied by one species only, *Nuttalliella namaqua* that occurs in Southern Africa [1]. Hard ticks feature a dorsal scutum, a hard chitinous plate that gives them their common name, which is lacking in soft ticks. Besides several other morphological differences between hard and soft ticks, there are also physiological and biological differences.

Ticks have four life stages and exist as egg, larva, nymph and adult. Whereas hard ticks have only one nymphal stage, soft ticks may have up to eight nymphal stages [2]. In general, soft ticks feed more frequently for a short time, minutes up to a few hours. They do not attach as firmly to their hosts as hard ticks and do not deposit a cement cone. Excess water is excreted *via* the coxal glands in soft ticks, while hard ticks use their salivary glands to excrete excess fluid during feeding with the saliva into the host. Female hard ticks take one large blood meal after which they lay their eggs and die, but female soft ticks may take multiple blood meals, laying smaller egg batches after each blood meal. Mating occurs off-host in soft ticks and on the host in hard ticks, with the exception of ticks belonging to the *Ixodes* genus that can also mate before feeding [3].

Ticks spend the greatest part of their lives off-host, for instance in the vegetation, leaf litter, soil or rock crevices, depending on the respective environment. They seek hosts for obtaining a bloodmeal and because they stay only on their host when feeding, ticks are described as temporary ectoparasites. These temporary stays can be of great medical and veterinary importance because ticks may affect their hosts, including humans, pets and livestock, in numerous ways.

First of all, the tick bite itself results in immune reactions, which occasionally can be more severe reactions, such as anaphylaxis [4] or red meat allergy [5]. Irritating itches, resulting in extensive grooming may lead to hair loss and accordingly decrease survival rates of certain hosts, such as moose, in winter [6]. Tick bites are also associated with secondary infections such as dermatophilosis in cattle, goats and sheep [7], which is also an economic problem because the skin condition impairs the quality of the leather. Heavy

infestation may be a life-threatening burden due to anaemia, especially for small, juvenile hosts [8]. The saliva from a number of tick species such as *Dermacentor andersoni, Ixodes rubicundus* and *Ixodes holocyclus* contains neurotoxins which can cause paralysis of the host [9]. But most importantly, ticks may act as vectors for a wide range of pathogens, including viruses, bacteria, and protozoa or even multicellular parasites like filariae [10].

Examples of tick-borne viruses of human medical importance include tick borne encephalitis virus (TBE), the causal agents of Crimean Congo Haemorrhagic Fever (CCHF), Omsk Haemorrhagic Fever, Colorado tick fever and Kyasanur Forest Disease. Lyme borreliosis, caused by *Borrelia burgdorferi sensu lato*, is probably the best known bacterial tick-borne disease. Other bacterial diseases transmitted by ticks include ehrlichiosis caused by *Ehrlichia chaffeensis,* human granulocytic anaplasmosis caused by *Anaplasma phagocytophilum*, tularemia caused by *Francisella tularensis* and Q-Fever caused by *Coxiella burnetii*. There are also several human pathogenic *Rickettsia* species, some of which cause spotted fevers or tick typhus, including *R. rickettsii, R. conorii, R. africae* and *R. sibirica*. *Babesia microti* and *B. divergens* are examples of zoonotic tick-borne protozoans.

Other tick-borne pathogens are of veterinary relevance, for instance protozoal parasites such as *Babesia bovis* and *B. bigemina,* the causal agents of bovine babesiosis, *B. canis* associated with canine babesiosis and *B. caballi*, which causes babesiosis in horses. Other relevant protozoans include *Theileria annulata, T. parva* and *T. equi*, the first two causing theileriosis in cattle and the third one in horses. *Hepatozoon canis* may cause hepatozoonosis in dogs. Bacterial agents include *Anaplasma marginale* and *A. ovis*, causing anaplasmosis in cattle and sheep, respectively and *Ehrlichia canis* and *E. ruminantum,* causing ehrlichiosis in dogs and cattle, respectively. Louping ill virus is an example of an encephalitis virus of veterinary importance, although it is a zoonosis and can also infect humans. Increased contact of humans with vectors, changes in land use but also progress in diagnosing and differentiating pathogens could lead to an increasing prevalence of tick-borne zoonoses in the future.

Soft ticks can transmit different *Borrelia* species, such as *B. duttoni, B. caucasica* and *B. turicatae*, to humans causing different forms of relapsing fever in different parts of the world. Several *Ornithodoros* species maintain African Swine Fever Virus in a sylvatic cycle

with wild suids, whereas contact of domesticated pigs with this virus leads to serious disease. They also transmit filariae of *Macdonaldius oschei* to snakes [10].

The work described in this thesis focused on two hard tick species in particular: *Ixodes ricinus* (Linné, 1758) and *Dermacentor reticulatus* (Fabricius, 1794).

Ixodes ricinus is the most widespread tick throughout Europe and can be found in a number of different habitats, from shady woodlands to open heathlands. Its activity varies during the year, avoiding winter cold and, depending on weather and habitat, the heat of the summer, which means that activity peaks are in spring and autumn, *videlicet* in moderate temperatures of about 10 – 20°C [11, 12]. *Ixodes ricinus* uses an ambush strategy in which it climbs on a blade of grass or spray of shrubbery and waits for a host to pass by. It can feed on a wide range of vertebrate hosts, including mammals, birds and reptiles [13]. Humans are a part of this spectrum and a number of pathogens can be transmitted to them. *Ixodes ricinus* is the most important European vector for *Borrelia burgdorferi sensu lato*, a species complex that may cause Lyme borreliosis, TBE virus associated with TBE and *Babesia divergens* which may cause human babesiosis [14]. *Ixods ricinus* males are smaller than females and have a dark scutum. Their mouthparts are short, about 300 µm, and they rarely attach to hosts. Females feature a reddish alloscutum with a dark scutum. They have long, protruding mouthparts with a length of approx. 550 µm. Engorged females are thought to resemble castor bean seeds, hence the common name ‘castor bean tick’.

Dermacentor reticulatus can be found in several parts of temperate Eurasia and its range in Europe is still expanding [15]. It is mainly found in the ecotone zone of forests, meadows, river valleys and waste land [16]. The adults are active over large parts of the year, thanks to their ability to withstand low temperatures, but may undergo diapause in winter. Peak questing activities are in April and September – October [17]. The scutum of *D. reticulatus* adults shows a pattern of light spots on a dark brown background, hence the common name ‘ornate dog tick’. The alloscutum is equally brown. *Dermacentor reticulatus* adults commonly feed on dogs, cattle and sheep, but can also be found on wildlife such as deer. Humans can occasionally be attacked; tick-borne lymphadenopathy (TIBOLA) is associated with *D. reticulatus* bites [18]. Adults of this tick also rely on an ambush strategy to find a host. Larvae and nymphs are endophilic – they stay in burrows of the rodents on which they feed, while the adults are exophilic [19].

Host finding and feeding

Ticks are able to locate their hosts using Haller's organ. This sensory organ is located on the first pair of legs and consists of an anterior pit and a posterior capsule, each filled with sensilla, with which the tick can sense carbon dioxide and host skin secretions. Radiant heat reception is also possible [20]. When sensing a nearby host, the tick starts waving its legs and holds fast as soon as physical contact with the host is established. Once on the host, the tick uses another chemosensory organ: the palpal organ, which is situated on the distal end of the palps and is brushed over the hairs or the skin surface to find a promising feeding site [3]. Once a promising site is located, the capitulum is pushed down to the skin. The chelicerae are pressed forward and cut through the skin, to facilitate the insertion of the hypostome into the skin at the feeding site. On the inner digits of the chelicerae, chemoreceptors (pit sensilla) are found that sense phagostimulants in the blood, including glucose, adenosine triphosphate (ATP), glutathion (GSH) and salts [21]. The tick may detach and reattach multiple times before finding the definite feeding site, to which it will bind firmly by the secretion of cement. Cement secretion is typical for hard ticks, although there are exceptions like *Ixodes holocyclus, I. pseudorasus* and *I. trianguliceps* that do not produce cement [20]. The cement is made by the salivary glands of the tick, a secretion consisting of approx. 82 % proteins and 18 % lipids, which hardens and forms a cone in the host epidermis. The shape and size of the cement cone varies amongst tick species [20].

The salivary glands also produce saliva, which contains a mixture of proteins with various functions. Tick saliva is antihemostatic to keep the blood of the host flowing and interferes with the immune system of the host using anti-inflammatory factors, complement inhibitors and modulators of host immunity [3]. For instance, the saliva of *I. scapularis* prevents neutrophil aggregation, T-cell activation and inactivates bradykinin and anaphylatoxin in natural hosts [22]. Furthermore, salivation is a form of excreting excess water and ions. Males also use saliva as lubricant for the transmission of spermatophores [23]. The glands grow in size during feeding to fulfil their task and degenerate after feeding when no longer needed [24]. Pathogens, too, are mainly transmitted to the host *via* the saliva, with some exceptions such as *Hepatozoon* spp.; infected ticks need to be ingested by the host during grooming so that mature oocysts containing infective sporozoites are set free in the lumen of the intestine and can penetrate the intestine wall [25], and *Coxiella burnetii* that may be

transmitted by inhalation of dust from sheep wool, possibly containing infectious tick faeces [26, 27].

There are many unsolved questions regarding the transmission of pathogens by ticks, the vector competence of different tick species and the interactions between tick, host and pathogen in general. To study this tick-host-pathogen triangular relationship, it is inevitable to use ticks in the laboratory, which includes their feeding on experimental animals. Screening for and testing of efficient drugs against ticks or their transmitted pathogens is another field of research and for these purposes, ticks are fed in the laboratory to maintain tick colonies. At the same time, scientists have the moral obligation to reduce animal experimentation and adhere to the 3R principle: the 'Replacement, Reduction and Refinement' of the use of experimental animals in research [28]. Consequently, artificial tick feeding methods have been developed, which ideally would result in a reduction in the number of experimental animals needed. Other reasons to feed ticks artificially are to lower expenses and their use in new research applications.
Successful *in vitro* feeding is well established for a number of different hematophagous arthropods, such as mosquitoes, tsetse flies and soft ticks [29, 30, 31, 32], but the *in vitro* feeding of hard ticks has proven to be more challenging. Reasons for this are their long feeding duration and their complicated host finding- and feeding behaviour, resulting in demanding attachment- and phagostimulant requirements. This explains why feeding success *in vitro* is typically lower than *in vivo* and the need for optimized *in vitro* feeding methods.

In the first reported attempts to artificially feed ticks, real animal skin [33] or batwing [34] was used as a feeding membrane, which the ticks had to penetrate to reach a heated blood source underneath. The advantage of this method is that real animal skin naturally provides all stimuli of a living host. Its main disadvantage is the decay of the skin, as it is in contact with the blood heated to body temperature, which may result in contamination of the blood. Consequently, this method was most successful for soft ticks as they have a short feeding period. Another approach to artificially feed hard ticks is by the use of baudruche membrane, which is made from the external layer (*serosa*) of cattle intestine. Baudruche membrane can be coated with glue or silicone to protect the organic material and thereby delay decay [35]. A disadvantage is that additional stimuli such as semiochemicals are required to make the membrane attractive to ticks. Parafilm, a stretchable polyethylene foil, is a simple version of an artificial membrane and has also

successfully been used to feed soft ticks [30]. The advantage of this method is that Parafilm is relatively cheap and easy to apply. The disadvantage is that it is not self-sealing and may leak during the long feeding period of hard ticks [36]. An alternative material for an artificial membrane is silicone. Silicone membranes have been developed and used for artificial hard tick feeding as well as feeding of other hematophagous arthropods such as mosquitoes and tsetse flies [29, 37, 38, 39]. Capillary feeding on the other hand is an alternative method to feed hard ticks *in vitro*, without the use of membranes [40]. Instead, the ticks' mouthparts are inserted into a capillary filled with blood, ready for the tick to imbibe. The advantage of this method is that only a minimum quantity of feeding blood is required. The main disadvantage is that experimental animals are still required to pre-feed the ticks, since it is not possible to feed ticks to repletion using this method. Capillary feeding is also laborious, since each tick has to be fed individually.

Silicone membranes, which were also used in the studies reported in this thesis, are cheap, durable, autoclavable and flexible enough to self-seal holes which are left behind following tick detachment. Thin membranes can be enforced by lens cleaning paper and attachment stimulants can also be added to mimic the skin of natural hosts, thereby increasing its attractiveness for ticks to feed on. These stimulants are important, because a silicone membrane by itself will not be attractive to ticks. At this point, the stimuli provided in an *in vitro* feeding assay are inferior to the natural stimuli of a live host, which results in lower feeding success compared to *in vivo* feeding.

Besides tactile stimuli and heat, semiochemicals may also stimulate attachment and feeding *in vitro*. Semiochemicals are emitted by organisms, bearing information for other organisms. In principle, there are two kinds of semiochemicals that can be used: pheromones and kairomones.

Pheromones are chemicals emitted by organisms to communicate with conspecific organisms, in this case ticks communicating with ticks. One of the most intensively studied group of tick pheromones are the aggregation-attachment pheromones of *Amblyomma* species. These are produced by dermal glands of feeding males and affect unfed males, unfed females, as well as unfed nymphs. Those are, as the name suggests, attracted to the pheromone and they tend to attach nearby the emitting tick, resulting in an aggregation of ticks on the host. These pheromones consist of different phenols like methyl salicylate, o-nitrophenol, 2,6-dichlorophenol and salicylaldehyd or alcohols like 1-

octen-3-ol and organic acids like pelargonic acid [41, 42, 43]. The triterpene squalene was found to be attractive to *Amblyomma americanum* as well as *Dermacentor variabilis* [44]. But tick faeces were also found to be attractive for conspecific ticks [45].

Kairomones are chemicals that are emitted by organisms of one species, received by organisms of another species and give an advantage to the receiver. In this case, any substance emitted by the host, enabling the tick to find the host, can be considered a kairomone. Skin gland secretions that define the body odour of the host are obvious kairomones for ticks and hence animal hair, hair extracts, sweat and ear wash concentrates have been used as attachment stimuli in artificial tick feeding [35, 38, 39, 46]. Also, breath components such as CO_2 are known to attract ticks, especially tick species that actively run towards potential hosts [47]. But even components of host faeces, such as uric acid in case of birds, have been reported to attract ticks as well [48].

All these semiochemicals are olfactory stimuli. But other senses of the ticks may be addressed as well to stimulate *in vitro* feeding, for instance by tactile stimuli, such as host animal hair or mosquito netting which provide texture to artificial membranes [46]. And there are phagostimulants such as ATP, GSH, glucose and 2,3-diphosphoglycerate which can be used as feeding blood supplements [39, 49].

The blood used for the *in vitro* feeding of ticks can be collected in large quantities at abattoirs during exsanguination. Bovine blood is an obvious choice as cattle is slaughtered regularly and its blood accrues in large amounts. It is also a natural host for many tick species of medical and veterinary importance such as *Ixodes ricinus, I. scapularis, Dermacentor reticulatus, Rhipicephalus microplus, R. appendiculatus, Amblyomma variegatum* and *A. hebraeum*. The blood can be stored refrigerated or frozen and defrosted before use [36] and other hematophagous arthropods such as tsetse-flies have been fed with oven dried blood which was rehydrated before use [50]. Coagulation is prevented by either manual defibrination [46] or supplementing an anticoagulant like heparin or sodium citrate [38]. Because blood is not gained in a sterile way at the slaughterhouse, it is necessary to limit further bacterial growth by irradiation or the use of antibiotics. Alternatively, blood can also be collected from donor animals. The advantage is that blood can be collected sterile, reducing the need for the use of antibiotics if all further artificial feeding steps are performed under sterile conditions. As this will still require the use of experimental animals, an artificial feeding medium would be an interesting

alternative, as this would also circumvent other problems like long-time storage and sterilisation of blood. Substitutes for feeding other hematophagous arthropods have already been developed [51, 52], but their use for feeding ticks has not been evaluated yet. The blood gets furthermore supplemented with glucose, which stabilizes erythrocytes as well as serves as a phagostimulant [21] and ATP or GSH as phagostimulants [21, 39]. It will be heated to body temperature during the feeding and therefore has to be changed regularly, usually twice daily [46] to prevent fouling, which will otherwise occur swiftly at that temperature.

Aims and outline of this thesis

The work described in this thesis forms a contribution to the optimization of *in vitro* hard tick feeding. The development of *in vitro* feeding methods that are as effective as *in vivo* feeding on animals would contribute to a reduction in the number of experimental animals required for this purpose. In **Chapter 2** effects on tick feeding and fecundity of different blood meal treatments (freezing, irradiation, addition of antibiotics), ambient conditions (increased CO_2 concentration) and phagostimulant use (addition of 2 g/L and 4 g/L glucose to the blood meal) were systematically evaluated. Both the efficacy of feeding and fecundity of engorged females are reduced compared to the results of natural feeding. This is why the optimization of these factors is crucial in order to replace tick feeding on animals by *in vitro* alternatives.

At the same time, a reduction in the manual labour associated with *in vitro* feeding is required. Several studies have focused on this part of the artificial feeding process for hematophagous arthropods [49-51]. In **Chapter 3**, a custom built semi-automated system was evaluated. Compared to the conventional system that was used before, it reduced manual blood changes form twice to once per day. Manual labour and close supervision are still necessary, but it is an important step in reducing the workload of artificial feeding.

Chapter 4 describes several experiments focusing on the optimization of artificial tick feeding, such as the *in vitro* feeding of *Amblyomma variegatum* as a representative tick species that uses a hunting strategy to find a host, the use of bioassays to identify potential attachment stimuli, a comparison between the phagostimulants ATP and GSH and an attempt to develop an artificial feeding medium for *D. reticulatus* ticks.

In summa, the work described in this thesis focused on ways to obtain better *in vitro* tick feeding results whilst limiting the workload associated with artificial feeding. In the future, an effective, practical *in vitro* system could become a platform for scientific applications, such as the *in vitro* infection of ticks, investigations into the vector competence of different tick species and the efficacy-screening of acaricides and anti-tick vaccine candidates.

Chapter 2: Optimization of an artificial tick feeding assay for *Dermacentor reticulatus*

Krull C*, Böhme B*, Clausen PH, Nijhof AM (2017)
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Christoph Krull performed all experiments in which the effects of increased CO_2 concentrations and increased glucose levels on tick feeding were evaluated, analysed these results and finally drafted the manuscript with Bettina Böhme and Ard Nijhof.

Chapter 3: Semi-automated in vitro feeding of *Dermacentor reticulatus* and *Ixodes ricinus*

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Christoph Krull performed all *I. ricinus* experiments, analysed these results and finally drafted the manuscript with Bettina Böhme and Ard Nijhof. It was published as “Evaluation of a semi-automated in vitro feeding system for *Dermacentor reticulatus* and *Ixodes ricinus* adults” under the DOI mentioned above.

Chapter 4: Additional experiments focusing on the optimization of artificial tick feeding

Besides the published work described in Chapters 2 and 3, several other experiments were performed with the aim of optimizing the *in vitro* feeding system. *Ambylomma variegatum* ticks were fed in efforts to examine the artificial feeding system for ticks other than *I. ricinus* and *D. reticulatus*. Different chemicals were tested in bioassays to identify possible attachment stimuli that could be used in combination with, or as an alternative to, hair extracts. The effects on tick feeding following the addition of the phagostimulants ATP or GSH to the blood meal were evaluated and an artificial feeding medium was tested for *D. reticulatus* ticks.

In vitro feeding of *Amblyomma variegatum*

Amblyomma variegatum (Fabricius, 1794) is a tick species native to Sub-Saharan Africa and was introduced to central eastern islands of the Caribbean with cattle shipped from West Africa in the 19th century where it subsequently became established [53]. It has a broad host spectrum including humans and it uses the so-called hunter strategy to find its host [54]. The hunting strategy involves an active approach of the host, which the tick locates by visual and chemical cues. This species is an important vector for heartwater disease caused by *Ehrlichia ruminantium* in domestic ruminants and African tick bite fever (*Rickettsia africae*) in humans. Infestations of *A. variegatum* are also associated with the occurrence of dermatophilosis (*Dermatophilus congolensis*) in animals.

The feeding system, the feeding blood and the feeding procedure used for this experiment are described in detail in Chapter 2, with the following modifications. Conspecific tick faeces was not added to the feeding units as it was not available at the start of the experiment. Bovine hair and hair extract were used for nymphs and horse hair and hair extract for adults due to availability. The glucose concentration in the feeding blood was 2 g/L and feeding units were kept in the incubator at 2.5 % CO_2, heated by the heating plate. *Amblyomma variegatum* nymphs originated from the *in vivo* colony of the Institute for Parasitology and Tropical Veterinary Medicine. The nymphs were fed in eight feeding units, using silicone membranes of 73-88 µm thickness. Twelve nymphs were put in each

feeding unit and fed for seven days. Thirty-tree nymphs engorged (33/96, 34.4%), of which six, four males and two females, moulted to the adult stage.

The feeding of *A. variegatum* adults *in vitro* is cumbersome since males must be pre-fed to release the aggregation-attachment-pheromone; females will only attach after this cue [42]. The pre-feeding period of the males *in vitro* lasted one week, after which the females were subsequently fed for another two weeks.
Two feeding units with silicone membranes of a 260 µm thickness were used, with two males and (after male pre-feeding of one week) one female in each unit. Only one of the females engorged successfully, with an engorgement mass of 1052 mg, which is relatively low for this species. By comparison, an average of 1.8 g for *in vitro* fed females of this species was previously reported by Kuhnert [55] and Garris even observed an average of 2.94 g after *in vivo* feeding [56].
Fungal infestation of the engorged female occurred after several weeks and killed the tick before it had laid any eggs.

Bioassays of possible attachment stimuli

In Chapters 2 and 3, the success of artificial tick feeding was evaluated through several parameters, including the engorgement rate and engorgement mass of fed ticks. These are critical parameters, since they entail other parameters such as mass of egg batch. It is therefore safe to state that the success of artificial tick feeding depends on how well the ticks accept the membrane as an artificial host and the feeding blood as a food source.
In order to make ticks accept the artificial and sterile environment of the glass feeding unit and silicone membrane, chemical stimuli are pivotal, since tick behaviour is largely influenced by receptions of chemosensory organs, as described earlier. A literature review suggests different stimuli for different tick species [41, 44, 48, 57, 58, 59]. We decided to test a selection of these chemical stimuli on *D. reticulatus* ticks, *videlicet* 2-nitrophenol, 2-salicylaldehyd, methyl salicylate, 2,6-dichlorophenol, uric acid, guanine hydrochloride and squalene (all Sigma-Aldrich). All chemicals are supposed to mimic either a host or other ticks to assemble with. In either case, ticks should be attracted. A bioassay, which was based on previous studies, was designed to test whether these chemicals indeed attract ticks [48, 59].

Round filter papers of 90 mm diameter (Sigma-Aldrich) were divided into quadrants by pencil strokes and a solution of the respective test substance in acetone (Sigma-Aldrich) was pipetted into the middle of two opposite quadrants (test). Pure acetone was pipetted into the middle of the other two quadrants (control). Concentrations of 0.1 M, 0.01 M and 0.001 M of each test substance were tested, with 25 µl of solution in each quadrant. Markings were made with pencil and nitrile gloves were worn throughout the preparation to prevent contamination with other odours. The filter papers were subsequently laid into plastic petri dishes of the same size (Sigma-Aldrich) and ten *D. reticulatus* ticks were placed into the middle of each dish. Males and females were evaluated separately to exclude potential confounding effects caused by emitted sexual pheromones. The petri dishes were closed and stacked into an incubator at 20 °C, 80 % RH, 2.5 % CO_2 and a 15 h : 9 h light : dark cycle. The topmost dish was covered with coloured paper to provide shade and a sand filled snap cover glass (ND22, Roth) on top for extra weight to guarantee that the petri dishes remained tightly closed. Ten petri dishes with ten ticks each were used, resulting in a total number of 100 ticks for each test substance. After 1 h and 24 h, the number of ticks in the test and control quadrants was counted. Additionally, a blend of equal quantities of all test substances mentioned above was tested. Likewise, a blend of all substances minus 2,6-dichlorophenol and 2-nitrophenol was tested. And as a positive control, horse hair extract at a concentration of 7 mg LVM/mL was tested, as it had successfully been used as an attachment stimulus in feeding experiments. In this case, DCM was the control substance, as it was the solvent used for the hair extract.
Each experiment was performed once, a statistical analysis cannot be given. After each experiment the ticks were kept in separate containers and were not used in bioassays again.

Table 1: Number of ticks (out of 100) counted in test quadrants

The test quadrants of the filter papers contain a solution of the substance to be tested, supposedly attractive to the ticks. Ticks were counted after 1 h and 24 h, separated by sex.

		♂ 1 h	♂ 24 h	♀ 1 h	♀ 24 h
A	squalene 0.1M	52	48	52	52
	0.01 M	51	52	45	45
	0.001 M	46	39	47	42
B	guanine hydrochloride 0.1 M	57	49	49	52
	0.01 M	43	37	49	48
	0.001 M	55	43	48	58
C	uric acid 0.1 M	71	56	50	56
	0.01 M	50	36	54	59
	0.001 M	44	63	36	39
D	2-salicylaldehyde 0.1 M	29	41	43	55
	0.01 M	57	54	49	41
	0.001 M	45	50	35	42
E	methyl salicylate 0.1 M	34	45	44	44
	0.01 M	39	38	51	31
	0.001 M	44	55	50	58
F	2,6-dichlorophenol 0.1 M	35	41	22	31
	0.01 M	30	32	27	37
	0.001 M	48	48	54	49
G	2-nitrophenol 0.1 M	19	28	34	29
	0.01 M	41	43	45	44
	0.001 M	55	45	39	34
	Blend of A - G 0.1 M	24	15	20	23
	0.01 M	23	12	27	30
	0.001 M	42	50	39	35
	Blend of A - E 0.1 M	41	38	33	29
	0.01 M	39	48	44	55
	0.001 M	30	40	36	38
	horse hair extract	63	68	54	68

ATP and GSH

Once ticks accept the membrane as an alternative to animal skin, the further success of artificial feeding depends on ticks accepting the feeding blood as a food source. For this reason phagostimulants, such as ATP or GSH, are added to the blood prior to feeding. Both were for instance used in combination with glucose in experiments on the artificial feeding of the soft tick *Ornithodorus tholozani* and were shown to induce a strong feeding response [60]. It was subsequently suggested that a gustatory chemoreceptor for GSH and ATP was present. Habedank et al. used ATP for the feeding of various tick species and added GSH if cattle blood was used [39].

To compare the effect of ATP and GSH on tick feeding, groups of *I. ricinus* ticks were simultaneously fed *in vitro* with 1 mmol/L ATP, 1 mmol/L GSH or 1 mmol/L ATP + 1 mmol/L GSH, each in combination with 2 g/L glucose. Two units with ten female ticks and ten male ticks each were used in every group, resulting in 20 female ticks per group.
The feeding took place in an incubator with 2.5 % CO_2 and feeding units were heated by a heating plate. All other conditions were similar to those described in Chapter 2.

The engorgement rates were 95 % (19/20) for ATP, 75 % (15/20) for GSH and 95 % (19/20) for ATP+GSH. The average mass of engorged females was 256.1 ± 59.5 mg for ATP, 310.7 ± 102 mg for GSH and 249.8 ± 94.5 mg for ATP+GSH (ANOVA P = 0.094). The portion of engorged females that produced fertile eggs was 36.8 % (7/19) for ATP, 6.7 % (1/15) for GSH and 31.6 % (6/19) for ATP+GSH. The average mass of egg batches was 49.6 ± 17.3 mg for ATP, 18 ± 0 mg for GSH and 33.7 ± 14.3 mg for ATP+GSH (Mann-Whitney-Test P =0.1). In the analysis of the egg batch mass, only one comparison could be made between the ATP and ATP+GSH group, since the GSH group only had a single value. Statistical tests were performed using SPSS software.

Artificial feeding medium

The development of a successful artificial feeding medium for ticks would greatly progress *in vitro* tick feeding; a standardized medium would facilitate reproducible experiments and results. Through sterilization, contamination that hinders the *in vitro* feeding would be avoided and storage stability would reduce the efforts of supply. There are therefore sufficient reasons to develop an artificial feeding medium for ticks, but an extensive

literature search did not reveal any. It was then decided to develop a feeding medium based on feeding media for mosquitoes and leeches [51, 61]. The initial medium consisted of 100 mL fetal calf serum (FCS) (Biochrom AG, Berlin, Germany), 600 mg NaCl (Roth), 60 mg $NaHCO_3$ (Roth), 3500 mg hemoglobin powder (Hb) (Roth) and 4 mg L-Arg (Roth). *Dermacentor reticulatus* ticks readily attached when offered this diet in a pilot experiment, but quickly showed abnormal swelling, which was thought to be caused by an osmotic effect. When the osmolarity of the medium was measured (Osmomat 030, gonotec, Berlin, Germany), it was indeed found to be too high, with 521 mOSm whereas pure FCS had an osmolarity of 236 mOsm, blood plasma of 333 mOsm and physiological saline of 281 mOsm. It was decided to remove NaCl and $NaHCO_3$ from the medium and a new feeding medium, which consisted of 100 mL FCS, 3500 mg Hb and 4 mg L-Arg, was prepared. For the following experiment four feeding units with seven female and five male *D. reticulatus* ticks were used. The feeding procedure used for this experiment is described in detail in Chapter 2, but instead of feeding blood the artificial medium was used and attachment stimuli consisted of horse hair and hair extract instead of bovine products. Feeding took place in the incubator with 2.5 % of CO_2 and the feeding medium was heated by the heating plate. Attached *D. reticulatus* ticks showed no abnormal swelling, but did not engorge fully (53.4 mg ± 17 mg), with an engorgement rate of 25 % (7/28). Two of the seven females (28.6%) produced fertile eggs, with egg batch masses of 10 and 11 mg.

Time of detachment

The experiments performed in Chapter 3 provided an opportunity to compare the time of detachment between *D. reticulatus* and *I. ricinus*. Of the 45 engorged *D. reticulatus* females, 31 (68.9 %) were found detached in the morning, and had thus detached in the previous night. The remaining 14 ticks (31.1 %) were found detached in the evening and had therefore detached during the day. Of the 115 engorged *I. ricinus* females, 17 (14.8 %) were found detached in the morning and 98 (85.2 %) were found detached in the evening.

Chapter 5: Summarizing discussion

Chapter 2

Several experiments were performed with *D. reticulatus* in order to optimize the *in vitro* feeding for this species and several facets of the artificial feeding process were examined in more detail. Dependence on the availability of blood through slaughterhouses and logistic efforts to collect feeding blood were one issue. It was tested whether blood can be stored frozen and then defrosted prior to feeding. In this way, larger amounts of blood could be collected at once and provide a supply that would last for several weeks. The results shown in Chapter 2 however suggest that refrigerating blood is preferable to the use of defrosted feeding blood. Although repletion and fecundity rates were comparable in both groups, the defrosted blood often showed fungal infestation, which in turn has adverse effects on tick feeding. The deterioration of blood is a general cause for concern, since it needs to be heated to body temperature during the experiment, which facilitates microbial growth. For this reason, sterilisation by irradiation and antibiotics was also investigated. The use of antibiotics proved to be better than sterilisation by irradiation, and resulted in a better tick fecundity. However, the use of antibiotics might not be the ideal solution, since the probable impact of antibiotics on tick microbiota is likely to impede long-term *in vitro* cultivation. The development of an artificial feeding medium that can be sterilised, might be an alternative solution here. Another aspect of *in vitro* feeding that could be optimized, is the repletion rate. It was therefore investigated whether a higher sugar concentration in the feeding blood would improve repletion. The standard of 2 g/L glucose was compared to 4 g/L and results indeed showed that feeding blood with 4 g/L of glucose had a tendency to increase the engorgement rates and masses. More concentrations and other types of sugar, such as trehalose, could be investigated in the future for further optimization purposes and also as additional components within an artificial feeding medium. An increase in the CO_2 concentration could also improve tick attachment, as carbon dioxide was shown to stimulate questing behaviour in ticks [47]. A 5 % CO_2–level was therefore compared to standard ambient conditions and the additional CO_2 indeed improved feeding results. Other concentrations could be tested in the future. During the bioassays in which possible attachment stimuli were evaluated (Chapter 4), many ticks dried out at 5 % of CO_2-levels, which is why the concentration was readjusted to 2.5 % for those experiments. The observed desiccation might be caused by water loss due to increased breathing activity under the stress of high CO_2 concentration, without the possibility to receive water from feeding blood. Especially for the feeding of immature

stages, which are usually more delicate in every way, lower CO_2 concentrations may be considered.
In general, the attachment rate is the factor that needs to be optimized first – not only for *D. reticulatus* larvae that did not attach successfully, but also for adults whose attachment rates needs to be improved in order to establish an effective *in vitro* cultivation on a larger scale. Attachment stimuli like CO_2 or hair extract are the method of choice; other stimuli may be identified in future studies.

Chapter 3

The amount of manual labour required for the regular blood changes is one of the major disadvantages of *in vitro* feeding, compared to *in vivo* feeding on natural hosts. To address this problem, a semi-automated feeding system (SAS), which reduced the number of daily blood changes from two to one, was developed and evaluated. Good results were achieved with this system for *D. reticulatus*, even improving the feeding success in the SAS compared to the conventional artificial feeding system. Results for *I. ricinus* were not as good, which might be explained by fungal infestation of the feeding blood that could spread rapidly within the SAS. Thus, sterility of the system should be prioritized in future experiments, for instance by disinfection of the ticks' mouthparts as these may also introduce microorganism into the blood meal. It should also be taken into consideration that the SAS uses more blood than the conventional feeding system, which could be a limiting factor for some applications. But the promising results for *D. reticulatus* suggest that further application and optimization of the SAS may be worthwhile. Of course, a fully automated system would be desirable for further reduction of manual labour and a sterile feeding medium may also improve sterility of the system.

Chapter 4

In vitro* feeding of *Amblyomma variegatum

The *in vitro* life cycle for the closely related species *Amblyomma hebraeum* was already described twenty years ago [62]. All life stages were fed *in vitro* successfully, however engorgement mass and percentage of females producing eggs were lower than in ticks fed *in vivo* and pre-oviposition times were longer. Ticks of this genus follow a hunting strategy to infest their hosts and their mouthparts are sufficiently long (about ½ cm) to penetrate a silicone membrane. These characteristics suggested that the *in vitro* feeding of *A. variegatum* would be relatively easy to achieve. However, in this study only a few

engorged nymphs moulted and only one adult female engorged, which did not lay any eggs after feeding. Considering the reduced fertility observed in the *in vitro* feeding of *A. hebraeum* by Kuhnert et al., a much higher number of ticks would have been necessary from the beginning to successfully establish an *in vitro* colony of *A. variegatum*. But that number of ticks was unfortunately not available.
The use of bovine or equine hair and hair extract in the feeding system was not thought to have influenced the results, since both vertebrates are natural hosts of this tick species. However, future experiments could be performed to compare the extracts from several host species and investigate the preferences of different life stages of the tick species in question.
During the feeding of *A. variegatum*, it was noticed that the silicone membranes tended to be more contaminated compared to feeding experiments with other tick species. This may be caused by the longer feeding duration or by a different bacterial flora harboured by this species. Surface sterilization of the tick, for instance with iodized alcohol or sodium hypochlorite [63, 64], could perhaps reduce the fouling and by that improve the quality of the feeding blood and consequently the feeding success. It could also prevent fungal infestation of engorged females.
At the same time, problems with the engorgement and egg fertility also occurred in the *in vivo* colonies of *A. variegatum*, not only at our institute but also at a colony from the same origin at the Slovak Academy of Sciences, Bratislava (personal correspondence with Dr. Maria Kazimirova). This suggests that the source of the fertility problem may not directly be related to the *in vitro* feeding system. Attempts to feed *A. variegatum* were however put to a halt hereafter and the focus was placed on *D. reticulatus* and *I. ricinus* instead. Most experiments were in fact performed with *D. reticulatus* for practical reasons. This species is abundant in Berlin/Brandenburg, is active over large parts of the year and can be flagged in large numbers. Moreover, adult *D. reticulatus* can be stored in the laboratory for prolonged periods of time without feeding.

Bioassays of possible attachment stimuli

The bioassays performed in this thesis were a pilot project to identify attractive substances. In case of noteworthy positive results more sets of 100 ticks each would have been tested in order to enable statistical analysis. Since such results were not found, it remained a pilot project. But nevertheless we can learn a few things from the findings made. *Dermacentor reticulatus* ticks seem to react indifferently to most of the chemicals tested. Some of the chemicals, such as 2-nitrophenol and 2,6-dichlorophenol even

seemed to work as a repellent, at least at the highest concentration tested. The reason for testing them as possible attachment stimuli was that they, as well as the other phenols tested, occur in the skin gland secretion of metastriate ticks [41, 65] and these secretions are, as mentioned before, believed to possibly function as aggregation pheromones and therefore attract ticks to the membrane. However, none of these reports are related to *D. reticulatus* specifically. Squalene was reported to be attractive to *D. variablilis* [44], which is at least the same genus, but was never before tested for *D. reticulatus*. Our data suggests that none of the substances tested are attractive to *D. reticulatus*, with the exception of horse hair extract, which was the positive control. This at least provided some evidence that the assay itself functions. *Dermacentor reticulatus* might rely on other semiochemicals and the substances tested here might be more effective for other species.

Another explanation for indifferent behaviour of the tested ticks towards the tested chemicals may be that natural secretions that work as stimuli are often complex blends of many different substances. An approach followed here was to test a blend of all the substances in equal portions, and then all the substances minus the two phenols that proved to be rather repellent in high concentrations. But the blends tested were not attractive either. In the future, starting from scratch by determining the skin gland secretion composition of *D. reticulatus*, followed by efforts to mimic the secretion with commercially available chemicals might be appropriate. It would be desirable to have attractants at least for the most important tick species to improve *in vitro* feeding, and it may turn out that these have to be tailor-made for each species.

Another aspect of this experiment concerns the concentration of CO_2. Initial experiments with 5 % of CO_2 led to the death of many ticks within 24 h. Apparently, dehydration might have been the cause, as discussed earlier, although the incubator was set to 80 % RH, which is the maximum setting. This subsequently resulted in resetting the CO_2 concentration to 2.5 %. If ticks really are under breathing stress at increased CO_2 levels, this might affect their behaviour, which is crucial for bioassays. Future studies may compare ticks' reactions to different substances at different CO_2 levels to investigate this question. And of course, different reactions to possible attachment stimuli at different CO_2 levels, and perhaps different behaviour in general, will also be of great interest for *in vitro* breeding of ticks in general.

ATP and GSH

Alternative use of ATP or GSH and the combined use of ATP+GSH as phagostimulants for *I. ricinus* ticks were evaluated in a small-scale experiment. Results show that the engorgement rate was highest in the ATP group and the ATP+GSH group. The mass of engorged females was highest in the GSH group, but they produced the least offspring. Instead, females of the ATP group produced the most offspring. In total, GSH was not found to be a superior stimulant compared to ATP and even the additional supplementation with GSH brought no clear advantage. Galun and Kindler reported a positive feeding response of the soft tick *Ornithodorus tholozani* to GSH [60], but this could not be confirmed for *I. ricinus* in this study. As a consequence, we did not proceed to investigate the use of GSH and continued using ATP as a standard supplement in the feeding blood for further experiments.

Artificial feeding medium

The use of bovine blood for the *in vitro* feeding of ticks is simple and effective. But it is not sterile when gained from slaughtered cattle during exsanguination. This poses a contamination source in subsequent tick feeding experiments and the use of antibiotics to limit bacterial growth may influence the tick microbiota, as mentioned previously. An alternative would be to collect blood aseptically. This would require the use of donor animals, which can be costly and is not entirely in line with the 3R principles for humane animal research. Accordingly, an artificial feeding medium would be an interesting alternative, if it could be standardized, sterilized and is storable. But blood is a complex medium and difficult to simulate, which is why FCS was used as the basis for an artificial tick feeding medium in this study. Although this may make sense from a practical point of view, FCS is also collected from animals and therefore not in agreement with the 3R principles. Although only a limited number of experiments were performed, it could be shown that it is possible to feed ticks to some extent on a blood substitute based on FCS. But the diet needs to be optimized extensively to obtain better feeding results. More desirable would be a completely artificial feeding medium to completely avoid the use of experimental animals or livestock. A cell culture medium could for instance form the foundation for this. It would need to be optimized regarding the nutrient requirements and also deliver appropriate phagostimulants to meet demands set by the sophisticated feeding behaviour of ticks. This means a lot of work, going far beyond the limits of this doctoral thesis, but it would mean great progress for *in vitro* tick feeding in the long run.

Time of detachment

The observed difference in preferred time of detachment for *D. reticulatus* and *I. ricinus* females is an interesting feature. Heylen and Matthysen observed a similar difference in the behaviour of the endophilic species *Ixodes abricola* (nightly detachment) and the exophilic species *I. ricinus*, which mostly detached during daytime [66]. The preference of *I. ricinus* to detach during daytime can be confirmed by data of this study, while *D. reticulatus* adults preferred to detach at night. But one must bear in mind that only two observations per day were made. So it is possible that a more complicated circadian rhythm is concealed here. Implying endophily as a cause for preferred times of detachment is problematic in this case, because *D. reticulatus* adults are as exophilic as *I. ricinus* adults. However, the immature stages of *D. reticulatus* are endophilic, living in burrows of rodents on which they feed. If preference of detachment time is indeed connected to endophily, this behaviour might be determined in the early life stages. But this is uncertain, since no data about detachment times of juveniles are available. Once the *in vitro* feeding of *D. reticulatus* juveniles is established, detachment data should be collected and analysed to pursue this matter. The use of a small, endoscope-like camera in the feeding unit could provide further information on tick behaviour such as attachment and detachment. Furthermore, investigations on a broader spectrum of tick species with known endophilic/ exophilic behaviour could deliver further data to analyse the connection between endophily and detachment time.

Outlook

The artificial feeding of ticks has come a long way since the first experiments which were performed over one hundred years ago and the work described in this thesis forms a further contribution to its improvement. However, the optimization is not complete yet as the results of natural feeding are still unmatched. It is therefore necessary to continue working on this topic and with all the different aspects of the ticks and the artificial feeding system the pestering question is: Where should we focus on? I believe that the most crucial parameter is the quantity of engorged ticks, which should be increased. The discovery or development of better attachment stimuli might be pivotal and olfactoric stimuli may be most efficient. However, there are plenty of other possibilities and facets to look at. The further development of defined artificial feeding media will for instance be important to make the method applicable for laboratories that have no immediate access to a slaughterhouse, and also to make experiments more comparable and robust, thereby

increasing their scientific value. Further automation will also be relevant, since the associated workload has a strong influence on the attractiveness of the method. The SAS is one method that shows potential, but there are many other possibilities as well. The use of robots for example, as they are already used for pipetting, might be an interesting approach in the future.

Summary

The *in vitro* feeding of hard ticks is a method that may be useful for studying different biological aspects of ticks and tick-borne diseases without the need for experimental animals. However, the development of standardized artificial feeding methods has been hampered by the complex feeding behaviour of hard ticks. The work described in this thesis therefore focused on the further optimization of artificial tick feeding methods using silicone membranes for the tick species *Dermacentor reticulatus* and *Ixodes ricinus*. Since the artificial feeding method requires regular laborious blood changes, the use of a semi-automated system (SAS) was also evaluated for feeding *D. reticulatus* and *I. ricinus* adults.

Various parameters were investigated in order to optimize the *in vitro* feeding of *D. reticulatus* adults, including different blood meal treatments (freezing, irradiation, addition of antibiotics), ambient conditions (increased CO_2 concentrations) and phagostimulant supplements (glucose, adenosine triphosphate (ATP) and glutathione (GSH)). In addition, various substances that were previously reported to be attractive to other hard tick species were tested in behavioural assays for their capability to attract *D. reticulatus*. This included different concentrations of 2-nitrophenol, 2-salicylaldehyd, methyl salicylate, 2,6-dichlorophenol, uric acid, guanine hydrochloride and squalene, with animal hair extract as a positive control.

Although fungal growth occurred more frequent in feeding units of ticks fed on defrosted blood, the attachment rate, engorgement mass and fecundity of females fed on defrosted blood did not significantly differ from that of ticks fed on fresh blood. A reduction in the fecundity of female *D. reticulatus* ticks was observed when ticks were fed with gamma-irradiated blood or untreated blood compared to blood treated with gentamycin. Both the engorgement mass and fecundity increased when ticks were fed at a 5% CO_2 level. A non-significant increase in the engorgement mass and engorgement rate of *D. reticulatus* was observed when blood was supplemented with 4 g glucose per liter compared to 2 g/l. The supplementation of blood with ATP and GSH had no significant effect on *I. ricinus* engorgement mass or fecundity. None of the tested substances was capable of attracting *D. reticulatus* adults in the behavioural assay.

Dermacentor reticulatus adults fed in the SAS obtained significantly higher engorgement masses and fertility rates compared to ticks fed in the conventional feeding system. In contrast, the engorgement rate and fecundity of SAS-fed *I. ricinus* were significantly

reduced in comparison to ticks fed in the conventional system, which was likely to be caused by fungal infestation that could spread between feeding chambers in the SAS.

Zusammenfassung

Die künstliche Fütterung von Schildzecken ist eine wichtige Methode, um wissenschaftliche Anwendungen zur Erforschung der Zecken und der durch sie übertragenen Krankheiten zu realisieren, ohne Versuchstiere verwenden zu müssen. Die Entwicklung standardisierter Fütterungsmethoden wird jedoch durch das komplexe Ernährungsverhalten der Zecken erschwert. Daher konzentriert sich diese Dissertation auf die Optimierung der Fütterungsmethoden der Arten *Ixodes ricinus* und *Dermacentor reticulatus* mithilfe von Silikonmembranen. Und weil die künstliche Fütterung regelmäßige arbeitsaufwändige Blutwechsel beinhaltet, wurde auch die Verwendung eines semiautomatischen Systems (SAS) für Adulte der genannten Spezies evaluiert.
Verschiedene Parameter wurden untersucht, um die künstliche Fütterung von adulten *D. reticulatus* zu optimieren. Diese Parameter betreffen die Behandlung des Blutes (Tieffrieren, Bestrahlen, Zugabe von Antibiotika), Umgebungsbedingungen (erhöhte CO_2 Konzentration) und die Zugabe von Phagostimulanzien (Glucose, Adenosintriphosphat (ATP) und Glutathion (GSH)). Es wurden auch verschiedene Substanzen, die auf andere Zeckenarten attraktiv wirken, auf ihre Attraktivität *D. reticulatus* gegenüber im Bioassay untersucht, namentlich verschiedene Konzentrationen von 2-Nitrophenol, 2-Salicylaldehyd, Salicylsäuremethylester, 2,6-Dichlorphenol, Harnsäure, Guaninhydrochlorid und Squalene, mit Tierhaarextrakt als Positivkontrolle.
Auch wenn in den Fütterungseinheiten mit aufgetautem Blut häufiger Pilzwachstum zu verzeichnen war, so gab es keinen signifikanten Unterschied die Vollsaugmasse und Fruchtbarkeit der Weibchen betreffend zu den Fütterungseinheiten mit frischem Blut. Die Fruchtbarkeit weiblicher *D. reticulatus* verringerte sich jedoch wenn die Zecken mit bestrahltem oder gänzlich unbehandeltem Blut gefüttert wurden, im Vergleich zur Fütterung mit Blut, das mit Gentamycin behandelt wurde. Vollsaugmasse und auch Fruchtbarkeit erhöhten sich wenn die Zecken bei 5 % CO_2 gefüttert wurden. Eine nicht signifikante Erhöhung der Vollsaugmasse und Vollsaugrate wurde beobachtet wenn das Blut mit 4 g/l Glucose versetzt war, im Vergleich zu 2 g/l. Die Zugabe von ATP oder GSH zum Futterblut hatte keine signifikanten Effekte auf die Vollsaugmasse und Fruchtbarkeit von *I. ricinus* Weibchen. Keine der im Bioassay getesteten Substanzen wirkte attraktiv auf *D. reticulatus*.
Adulte *D. reticulatus*, die im SAS gefüttert wurden, wiesen signifikant höhere Vollsaugmassen und Fruchtbarkeit auf als jene, die im konventionellen System gefüttert wurden. Vollsaugrate und Fruchtbarkeit der im SAS gefütterten *I. ricinus* waren jedoch

signifikant geringer als die der konventionell gefütterten Zecken, was vermutlich durch Pilzwachstum zu erklären ist, das sich zwischen den Kammern des SAS ausbreiten konnte.

Publications and presentations

Publications:

Böhme B, Krull C, Clausen PH, Nijhof AM (2014) In vitro feeding of *Dermacentor reticulatus*. Workshop on Ticks and Tick-borne Diseases, Berlin, 30.09.-02.10.2014, p59. ISBN 978-3-00-047198-8

Krull C, Böhme B, Clausen PH, Nijhof AM (2017) Optimization of an artificial tick feeding assay for *Dermacentor reticulatus*. Parasit Vectors 10(1):60

Böhme B, Krull C, Clausen PH, Nijhof AM (2018) Evaluation of a semi-automated in vitro feeding system for *Dermacentor reticulatus* and *Ixodes ricinus* adults. Parasitol Res: 1-6

Presentations:

Böhme B, Krull C, Clausen PH, Nijhof AM (2014) OAKS: optimization and automation of artificial tick feeding. Joint 8th International Ticks and Tick-borne Pathogens (TTP8) and 12th Biennial Society for Tropical Veterinary Medicine (STVM) Conference, Cape Town, South Africa, 24.-29.08.2014

Nijhof AM, Böhme B, Krull C (2015) Artificial feeding and infection of ticks. Workshop on Ticks and Tick-borne Diseases, Český Krumlov, Czech Republic, 26.-27.03.2015

27th Annual Meeting of the German Society for Parasitology, Gießen, Germany,09.-12.03.2016 in (Poster & Turbo Talk)

Krull C, Böhme B, Pröhl S, Nijhof AM (2016) Artificial feeding of *Ixodes ricinus* and *Dermacentor reticulatus* ticks. Tagung der DVG-Fachgruppe “Parasitologie und parasitäre Krankheiten”, Berlin, Germany 02.-04.05.2016

Krull C, Böhme B, Pröhl S, Nijhof AM (2016) Artificial feeding of *Ixodes ricinus* and *Dermacentor reticulatus* ticks. 8th Symposium of the European Association of Acarologists (EURAAC), Valencia, Spain, 11.-15.07.2016

First Joint International Conference of the Association of Institutions for Tropical Veterinary Medicine (AITVM) and the Society of Tropical Veterinary Medicine (STVM), Berlin, Germany, 04.-08.09.2016 (Poster)

Krull C, Böhme B, Nijhof (2017) Artificial tick feeding and its potential use in acaricide efficacy screening. 18th Drug Design & Development Seminar (DDDS), Borstel, Germany, 30.-31.03.2017

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Erklärung

Hiermit erkläre ich, dass ich die vorliegende Arbeit ohne die unzulässige Hilfe Dritter und ohne die Verwendung anderer als der angegebenen Hilfsmittel angefertigt habe.
Diese Arbeit wurde bisher weder im In- noch Ausland in gleicher oder ähnlicher Form einer anderen Prüfungsbehörde vorgelegt.

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